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Weatherability of a steam-rolled oat groat chlorophacinone ground squirrel bait under field and laboratory conditions

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Abstract

Investigations of the weatherability of spot-baiting for ground squirrel control in northern CA field studies were conducted with a registered bait consisting of 0.01% chlorophacinone (an anticoagulant rodenticide) on steam-rolled oat groats. For reference purposes, a laboratory test was later conducted in an environmental chamber simulating some of the observed weather conditions. Three weathering plots were established in alfalfa for field tests. Each was baited with rodenticide fortified bait that was handled the same as for a simultaneous control project. Test areas were protected with wire mesh to prevent bait consumption by birds and mammals. Bait samples were collected daily over 7 days, then frozen, and shipped for analysis. Test No. 1 conducted under wet conditions showed a 71% loss of chlorophacinone after 1 week. Test No. 2 demonstrated a 57% loss of chlorophacinone under drier conditions. Test No. 3, a 24 h test under very wet conditions within the alfalfa field irrigated by overhead sprinklers, had a 92% loss of chlorophacinone. Laboratory studies using controlled environmental conditions: light (16 h light:8 h dark), with a mean relative humidity of 98%, and a range of temperature 11.1–27.8°C (52–82°F) showed ≈50% loss of the chlorophacinone. The magnitude was less (37% at 7 days) in the environmental chamber when corrected for water weight gain. Published by Elsevier Science Ltd.

1. Introduction

Chlorophacinone (2-[(*p*-chlorophenyl) phenylacetyl]-1,3-indandione) is one of the most potent of the first generation anticoagulants. It has a long history of use as a multiple dose rodenticide in commensal rodents (Gill, 1992), and later it's use included control of field rodents (e.g., ground squirrel control in CA, Clark, 1978). Rodents usually consume the bait over a period of a week or more. Because its use does not produce bait shyness (Marsh, 1994), chlorophacinone can be used whenever rodents are active and consuming seeds (Passof, 1974). Many field studies, such as Sauer (1976) using chlorophacinone to control rodents do not indicate the degradation of the baits by weather (light, temperature and precipitation), physical loss (handling and application), or loss from sprinkler irrigation; however, some do analyze some aspects of this field of research (Sterner and Ramsey, 1995). In association with studies comparing 0.01 and 0.005% chlorophacinone steam-rolled oat (SRO) groat baits to control Belding's ground squirrels (*Spermophilus beldingi*), a separate investigation of bait weatherability under spring operational conditions in alfalfa (*Medicago sativa*) was completed. It included the customary use of overhead sprinklers by alfalfa cooperators. We followed this with a laboratory study to determine whether the observed loss of chlorophacinone was due to some of the observed environmental conditions.

2. Study site

The field study was conducted within the range of Belding's ground squirrels in northern CA, Siskiyou County, in the north-east corner of the Butte Valley. The valley is ≈17 miles long and 12 miles wide

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(USDA, 1994). The study site, with pivoting overhead sprinklers were used to irrigate the alfalfa field, was located approximately 1 mile south of Dorris (T47N, R1W, Section 1) with an average elevation of 1230 m (4035 ft). The surface soils were predominantly Poman-Fordney (fine sandy loam), with the native vegetation consisting of perennial grasses, shrubs, and forbs. The major agricultural management concern in the area was rapid water loss; therefore, overhead-sprinkler irrigation was the most commonly used method of watering the fields.

The climate in the Butte Valley is tempered by winds from the Pacific Ocean. Summers are fairly warm with hot days being rare; winters are cold and snowy, with freezing temperatures common. The average annual precipitation is ≈30.5 cm (12 in.) and mean annual temperature ≈8.9°C (48°F). The average frost-free period is ≈50 days. The average temperature during the 26-day study was 18.3°C (65°F) with an average minimum temperature of 2.2°C (36°F). A total of 4.44 cm (1.75 in.) of precipitation fell during the study. The Butte Valley's location, topography, soil, vegetation, and arid conditions make it suitable for livestock grazing, and for production of alfalfa and various cultivated crops including wheat, barley, oats, and potatoes.

The California Department of Food and Agriculture (CDFA) has a state registration with the United States Environmental Protection Agency (EPA) for the use of chlorophacinone in controlling California rangeland rodents, including ground squirrels (*Spermophilus beecheyi* and *S. beldingi*), pocket gophers (*Thomomys* spp.), deer mice (*Peromyscus* spp.), and house mice (*Mus musculus*). The EPA recommended that efficacy data be developed for the reregistration of 0.01% chlorophacinone grain bait (EPA SLN CA-890024) for use by hand-baiting or in bait stations for control of Belding's ground squirrels in alfalfa. Associated with the EPA-recommended study reported elsewhere, 0.01% chlorophacinone bait degradation was investigated in areas adjacent to the field plots and also under controlled laboratory conditions to further understand the field results.

3. Materials and methods

3.1. Chlorophacinone baits

The 0.01% chlorophacinone and 0.0% control baits were formulated by Rodent Control Outfitters of Monroe, OR, following the procedure outlined in the Confidential Statement of Formulation for EPA SLN CA-890024 and Vertebrate Pest Control Handbook (Clark, 1986). Chlorophacinone (CAS No. 3691-35-8) was applied as a dry powder to SRO groats (squirrel type thickness) using Alcolec-S, an adhesive. The assays indicated the mean percent of chlorophacinone observed (w/w) was 0.0109% (SD ± 0.00008%) for the nominal 0.01% bait. The untreated control bait assayed at 0.0% (SD ± 0.0000%).

Bait application was made according to label specifications for EPA SLN CA890024. Dippers (small cups with handles) were used to dispense 225 g (≈0.5 lb) of 0.01% chlorophacinone bait evenly over 1 m^2. Rubber gloves and protective clothing were worn by all study participants. California regulations required that bait applicators wear long sleeve shirts, splash-proof or dust-resistant safety goggles, and gloves to prevent any possibility of skin contact with the pesticide.

3.2. Bait environmental weatherability

Three environmental weatherability plots were established in the alfalfa. Each plot consisted of nominal 5.08 cm × 15.24 cm (2 in. × 6 in.) lumber to build the perimeter of a 1 m^2 study area. The 1 m^2 areas were baited with 0.01% treated bait but with differing weather conditions, but similar handling. Each 1 m^2 frame was covered by tightly stretched chicken wire (≈1.2 cm oval) to protect the baits from birds and mammals. Samples of 25 oat groats were taken daily (≈17:30 pm) from each 1 m^2 area. Each sample was handled with rubber gloves, immediately placed into a labeled plastic bag and sealed, and stored in cooler in the field until transported to the freezer (≈30 min). Samples remained in the freezer from 1 to 21 days before being shipped on dry ice to NWRC's analytical chemistry project (ACP) laboratory where they were stored in freezers until the residue analyses were done (storage stability tests have shown the bait to be stable at room temperature for up to 1 year, B.E. Petersen, NWRC, personal communication). For Test Plot No. 1, treated samples collected on May 13 (day 0), 15, 19, and 26, 1996 were analyzed for the amount of 0.01% chlorophacinone remaining with the baits under these "wet/rain" weather conditions (9 days of rain totaling 1.6 in.). These samples were analyzed on September 6, 1996. A second weatherability series, Test No. 2, was initiated on May 19, 1996, after a early morning rain (0.02 in.). These chlorophacinone samples were taken during "dry/no rain" conditions on May 21 (day 0), 23, 25, 27 and 30, 1996 with rain on May 27 (0.16 in.). These samples, handled in the same way as those for Test No. 1, were analyzed on October 4, 1996. Test No. 3 was similar in the setup and details to Tests No. 1 and 2, but sampling was conducted 24 h after baiting placement on May 30, 1996. This test plot was positioned in alfalfa under the overhead sprinkling system that distributed 3.9 in. of water over the baits during 24 h (measured by a rain gauge positioned near the center of the treated 1 m^2 area) before moving on.

During these experiments the temperature ranged from −2.2°C minimum to a 27.8°C maximum (28–82°F); daily precipitation from May 13 to May 30 ranged from 0.0 cm (0 in.) to 0.91 cm (0.36 in.), with rain occurring on days May 14–22 and 27 as shown in Table 1.

3.3. Bait environmental weathering under laboratory conditions

Field samples were not evaluated for the effect of water absorption or mold growth on the magnitude of chlorophacinone weathering. The weathering of chlorophacinone on SRO baits was studied in a Revco model PG-8-1045-A environmental chamber at 27.8°C (82°F) for a day period of 16 h and 11.1°C (52°F) for the night period of 8 h with an average relative humidity maintained at 98%. Each sample was placed in a glass petri dish and pre-weighed. The samples were positioned randomly in the chamber 0.3 m from a bank of fluorescent and incandescent light bulbs. Weathered bait samples were removed on days 0, 1, 3, 5, 7, and 10. Samples were then ground and prepared for chlorophacinone analysis (Primus et al., 1998).

Table 1
Climatic conditions at the Dorris study site, May 1996

Study day	Date	Temperature (°F)		Precipitation[a] (in.)
		Minimum	Maximum	
1	5–4	22	60	–
2	5–5	32	61	–
3	5–6	27	65	–
4	5–7	30	63	–
5	5–8	27	63	–
6	5–9	25	66	–
7	5–10	28	72	–
8	5–11	35	80	–
9	5–12	45	82	–
10	5–13	46	80	–
11	5–14	48	69	0.27
12	5–15	47	58	0.36
13	5–16	43	54	0.28
14	5–17	44	54	0.07
15	5–18	39	54	0.19
16	5–19	34	55	0.05
17	5–20	28	64	0.01
18	5–21	41	64	0.34
19	5–22	32	54	0.02
20	5–23	27	52	–
21	5–24	34	64	–
22	5–25	41	72	–
23	5–26	41	73	–
24	5–27	43	72	0.16
25	5–28	32	64	–
26	5–29	30	63	–
27	5–30	30	64	–
28	5–31	36	69	–

[a] Precipitation is recorded for the previous 24 h period.

3.4. General laboratory method

Several grams of each sample were ground with an electric coffee mill (Krupp, Type 203B) and 1.00 g aliquots were weighed into a 50 ml polypropylene tube and extracted with 10.0 ml of a 5 mM potassium tetrabutylammonium phosphate solution in methanol. The samples were vortexed for 10 s, shaken on a mechanical shaker for 10 min, and sonicated for 20 min. All samples were analyzed in triplicate. The samples were filtered and analyzed with a 1090M Hewlett-Packard (Palo Alto, CA) high performance liquid chromatograph (HPLC) equipped with a diode array detector and controlled by a computer work station (Primus et al., 1998). Several sample extracts were screened for chlorophacinone degradates by high performance liquid chromatography/mass spectrometer (HPLC/MS) using a Finnigan LCQ (Palm Beach, FL).

4. Results

Recovery of chlorophacinone on SRO groats for field and lab samples was $92.2 \pm 1.7\%$ ($n = 12$). Bait environmental weathering/exposure characteristics are shown in Fig. 1. Test No. 1 conducted under "wet/rainy" conditions showed a mean loss of chlorophacinone $\approx 71\%$ at 1 week; baits in Test No. 2 showed a mean loss of chlorophacinone of 57% during the initial 7 days of the test under "dry" conditions. Tests No. 1 and 2 both showed similar slope for the loss of chlorophacinone during the initial 3 days of exposure, with $>50\%$ of the 0.01% chlorophacinone lost. Test No. 3, a 24 h test under very wet conditions (i.e., overhead sprinkler set to maximize alfalfa growth), showed a much greater loss (i.e., $>90\%$) of chlorophacinone. The weathered chlorophacinone field and lab samples data are shown in Fig. 1. These samples were not checked for mass changes based on the absorption of water or the growth of molds. A dark colored mold or

Table 2
Light conditions during the laboratory study

Sensor[a] spectral response range (nm)	Maximum sensitivity (nm)	Intensity ($\mu W/cm^2$)
240–320	275	13–17
260–360	310	240–280
310–390	360	315–480

[a] For each of the sensors, in the order listed above, the light intensity for a cloudy day in Colorado at an altitude of 4900 ft in late July was 14, 178 and 350 $\mu W/cm^2$.

fungi was observed on the samples collected after 5 days of exposure in the field.

In the environmental chamber, chlorophacinone weathering was confirmed (≈50% for 7 days), but the magnitude was up to 18% less at 7 days compared to degradation observed under field conditions for the time period (Test No. 1 and 2). The light intensity was measured occasionally during the lab experiment and the range recorded (Table 2). Samples were removed from the chamber and weighed. Weight gain of each sample was used to correct for the apparent chlorophacinone concentration as water mass accumulated by the bait would make the chlorophacinone appear to weather at a higher rate. The sample weight gain during the initial 4 days of study was mostly absorption of water. On day 5, the growth of mold and slime commensed in very wet samples. As observed in the weathering of the chlorophacinone on the field-weathered baits, the loss was apparent (37% less at 7 days) even when the sample weight gain was corrected for water absorption.

With chromatograms of sample extracts as shown in Fig. 2, it can be demonstrated that extracts of samples become more complex with time, especially with

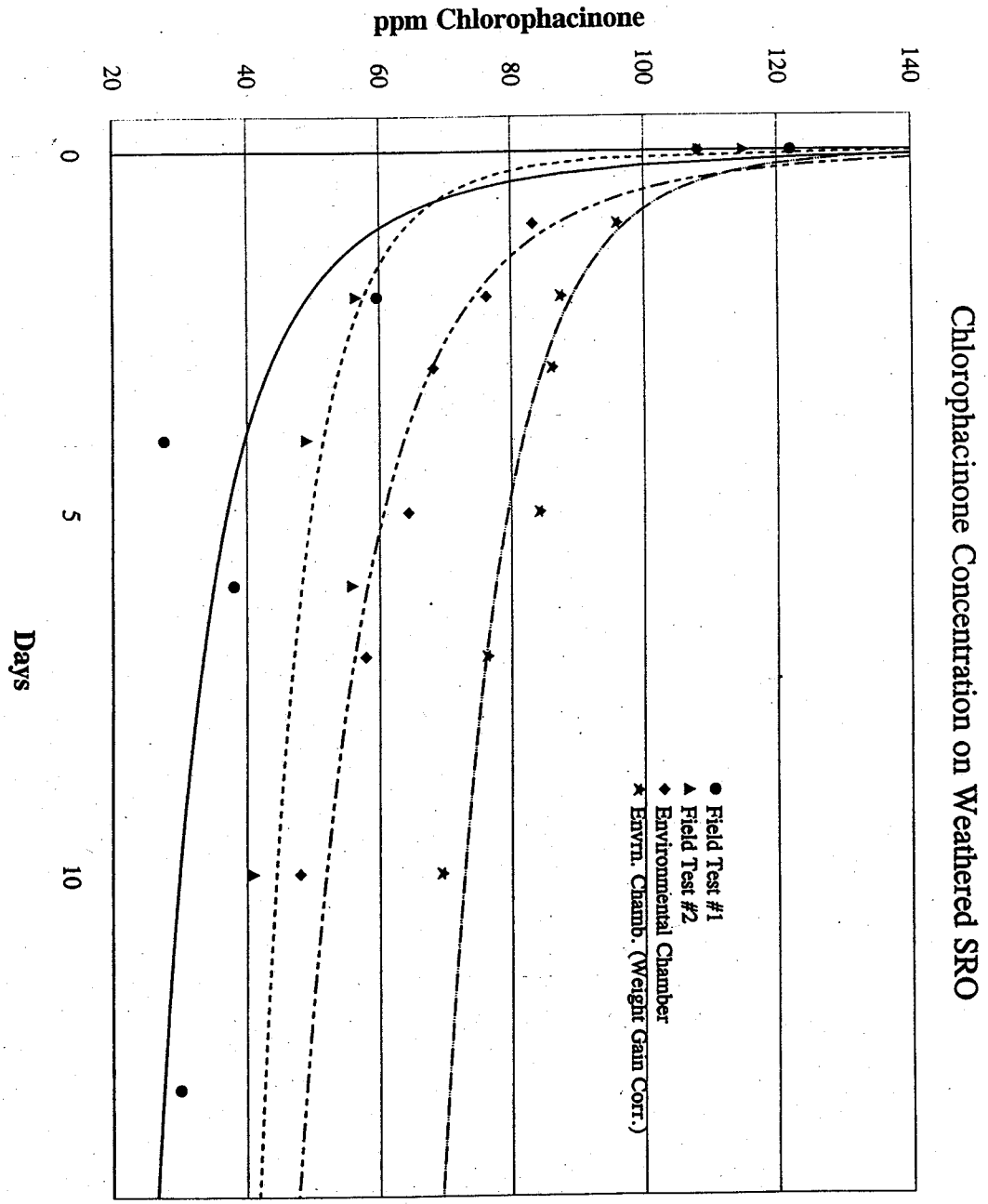

Fig. 1. A comparison of the degradation of chlorophacinone over time for each of the experiments.

increased water content, in addition to the decreased concentration of chlorophacinone. An attempt to identify degradates was pursued as previous work with chlorophacinone during photodegradation in water and soil has shown that *o*-phthalic acid and *p*-chlorophenylphenyl acetic acid are formed (Spare, 1992), at least temporarily. With ion-pairing reversed-phase chromatography, reversed-phase chromatography, and HPLC/MS with electro spray ionization while infusing the sample, neither of these degradates could be identified.

5. Discussion

The apparent loss of chlorophacinone from the baits even after correcting for water weight gain, could possibly be due to photodegradation or sloughing of the surface-coated chlorophacinone (Pank, 1976) due to rainfall or the handling of the baits. In both field and lab studies, the loss of chlorophacinone was significant (> 50%) after 7 days before significant mold and slime growth was observed under rainy and dry conditions. Such losses justify the use of 0.01% baits for spot application, and the weathering of residual bait especially under rainy conditions, once desired control has been achieved, logically offers some safeguard to non-target species. For application under overhead sprinkler irrigation, the bait lost significant potency ≈90% after 24 h. This new finding suggests bait placement should not to be in the line of the overhead sprinklers for several days.

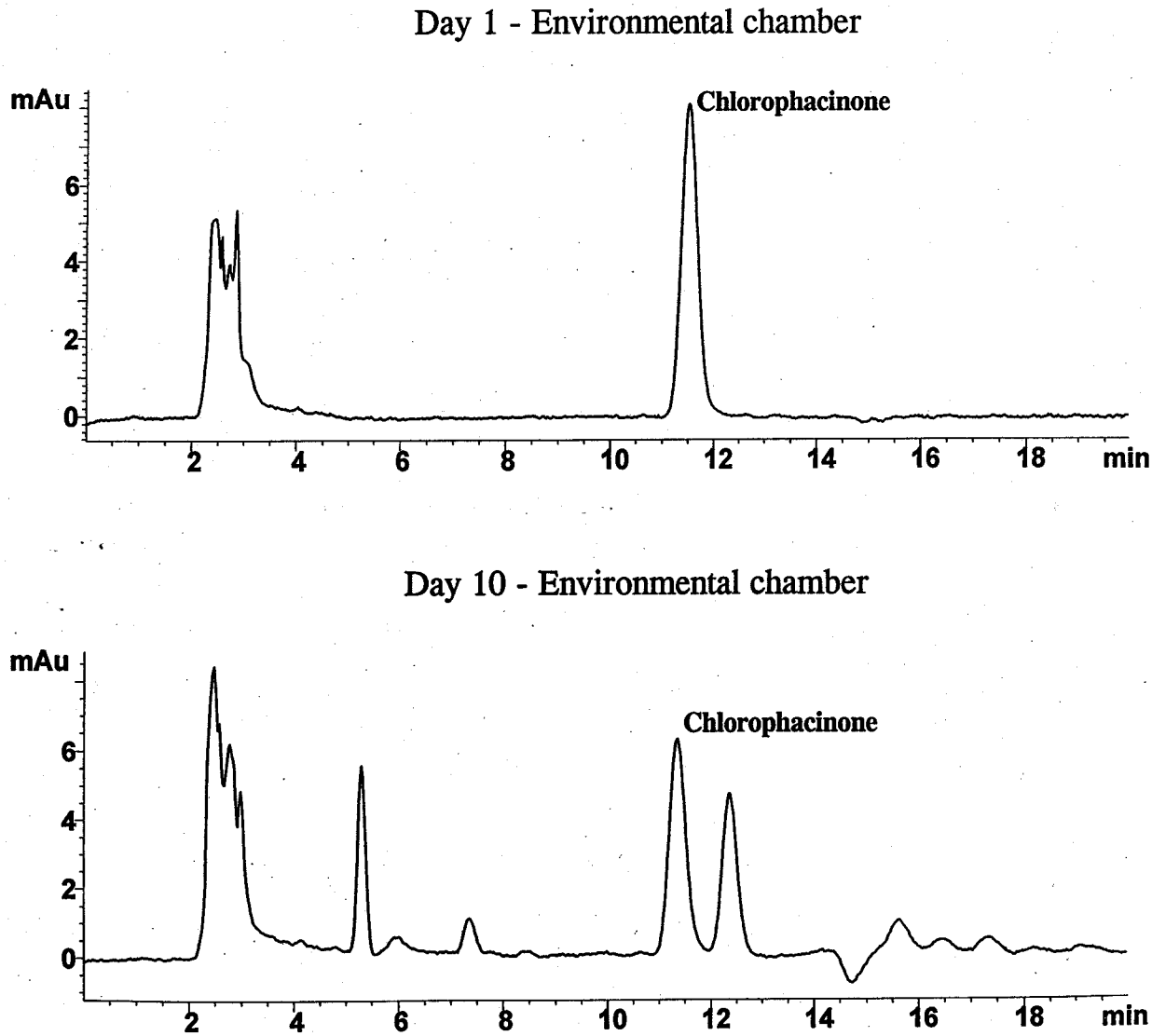

Fig. 2. Chromatograms of sample extracts from day 1 and day 10 samples exposed in the environmental chamber.

A concurrent study using hand baiting with these same 0.01% chlorophacinone on SRO groat baits (Ramey et al., 1999) could probably have achieved even greater control (as measured directly by visual counts — 64% and indirectly by the open-hole index — 68%) by putting out less bait each baiting day (>50% remained after 48 h based upon visual inspection) and increasing the number of baiting days to include a third day, 96 h after the initial baiting. This would probably expose the bait to less UV light, weather, and other types of environmental degradation as suggested by Askham (1986), and thus increase its efficacy.

Regarding the hazard to non-targets, Stimman and Clark (1981) suggested, based on general observations, that under spring operational use non-target mortality should be minimal with chlorophacinone. The 0.01% chlorophacinone baits used in the current study would appear to retain sufficient potency under spring conditions to be both efficacious (>64%) and relatively safe for non-target species when applied in fields above ground. Further research should be undertaken to develop the ideal oat bait for ground squirrels.

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